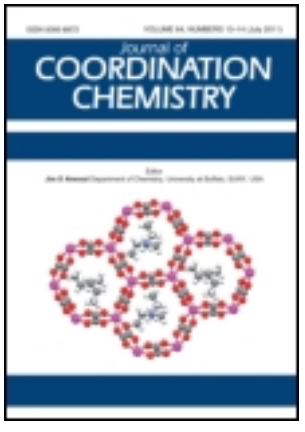

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Synthesis, characterization, and *in vitro* antibacterial and antifungal studies of tin(IV) thiohydrazide complexes

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Reaction of tin dichloride and tin tetrachloride with cyclohexylamine-N-thiohydrazide (ChaThz) [L^1] and 1,3-propanediamine-N-thiohydrazide (PdaThz) [L^2] results in $[Sn(ChaThz)_2]$ (**1**), $Sn(ChaThz)_2Cl_2$] (**2**), $[Sn(PdaThz)_2]$ (**3**), and $[Sn(PdaThz)_2Cl_2]$ (**4**), in which the thiohydrazide coordinates to tin through imine nitrogen and thioamide sulfur. The ratio metal : ligand was 1 : 2 for all complexes. The tin(IV) thiohydrazide complexes were characterized by elemental analysis, IR, UV-Vis, ^{1}H-NMR, ^{119}Sn NMR, and mass spectral studies. Using the disc diffusion method, the ligands and metal complexes were screened for *in vitro* antibacterial activities against four pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus*, *P. aeruginosa*, and *Bacillus cereus* and for antifungal activities against *Aspergillus flavus*, *A. carbonarius*, *A. niger*, and *A. fumigatus*. While the tin(IV) complexes exhibited moderate antifungal activities, their parent ligands showed much higher and long-lasting broad spectrum of bioactivity against fungal growth. This was particularly the case for L^1 whose fungal inhibitory activity by the end of the experimental period was comparable and, for the most part, more pronounced than that of AmB. This higher activity of L^1 was maintained specifically against *S. Aureus* but in general, bacteria were more susceptible to complexes than ligands.

Keywords: Synthesis; Thiohydrazides; Tin(IV) complexes; Antifungal; Antibacterial

1. Introduction

Tin compounds have been widely studied due to their numerous applications as antifungal, antibacterial, biocidal, and cytotoxic agents [1, 2]; toxic behavior of tin compounds has also been reported [3, 4]. Therefore, the synthesis of tin complexes with various nitrogen and sulfur donors could be a strategy to prepare new compounds with promising pharmacological properties.

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Some organotin(IV) complexes with N(4)-phenyl-2-benzoylpyridine thiosemicarbazone exhibit cytotoxic activity against various human tumor cell lines [5]. Compounds containing thione and thiol groups show potential donor properties through transition metal ions [6]. Organic compounds as well as metal complexes display a wide range of pharmacological activities including anticancer, antibacterial, and fungicidal effects. Coordination chemistry of thiohydrazide, thiodiamine, and thiohydrazone has been reported [7–9]. Compounds containing thiol groups are easily converted to the corresponding methyl sulfides (methyl thioethers) and ligands with sulfur and nitrogen donors in their structures are good chelating agents for transition and non-transition metal ions [5–11]. Coordination of thiohydrazide, thiodiamine, and thiohydrazone compounds with transition metal ions often enhances their activities against microorganisms [9, 10].

Interest in transition metal complexes of such ligands covers several areas ranging from general considerations of the effect of sulfur and electron delocalization in transition metal complexes to potential biological activity and practical applications [12]. Several bioactive agents including ligands and transition metal complexes [7, 13–15] have been synthesized, tested, and found to be potentially active against fungi and bacteria. However, antifungal properties of complexes have not been studied over time to determine whether such activity is long-lasting.

This study focuses on the synthesis, characterization, and antimicrobial (biological) studies of tin(IV) thiohydrazide complexes. The synthesized ligands and the complexes were characterized by elemental analysis, IR, electronic, ^{1}H-NMR, ^{119}Sn-NMR, and Mass spectroscopic studies. Molar conductivity was determined for these complexes. *In vitro* antibacterial and antifungal studies were also carried out for these ligands and their metal complexes.

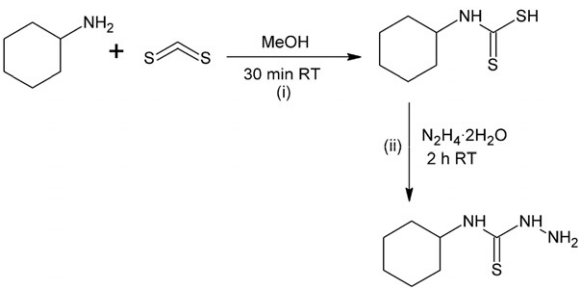
2. Experimental

2.1. *Materials*

All reagents and solvents used were of analytical reagent (AR) grade. The stannous chloride, stannic chloride, cyclohexylamine, hydrazine hydrate, and 1,3-propanediamine of synthetic grade was procured from Merck South Africa. Carbon disulfide was obtained from Sigma-Aldrich, South Africa.

2.2. *Instrumentation*

IR spectra were recorded on a MIDAC FTIR spectrophotometer from 4000 to 400 cm^{-1} using KBr pellets. The UV-Vis (electronic) spectra were recorded in dimethyl sulfoxide (DMSO) on a Cary 50 Perkin Elmer UV-Vis spectrophotometer. ^{1}H NMR spectra were recorded on a GEMINI-300 MHz spectrophotometer at room temperature using DMSO-d_6 as a solvent. ^{119}Sn NMR spectra were recorded on an INOVA-300 at 111.85 MHz in DMSO-d_6 as solvent at 50°C, power 39 dB, relax delay 3 s, pulse 54.8° and width 167.7 KHz parameters. Elemental analysis was performed on a Thermo Fisher Scientific CHNS/O Analyser Model Flash 2000. Mass spectra were recorded on a Waters 3100 Mass detector Tune Parameters – Empower Pro by



Scheme 1. Synthesis scheme for cyclohexylamine-N-thiohydrazide (ChaThz), L^1.

direct injection. Melting points were measured using an Electrothermal Digital Melting Point Apparatus and molar conductivity was measured on a Crison EC Meter BASIC 30+.

2.3. *Preparation of thiohydrazides*

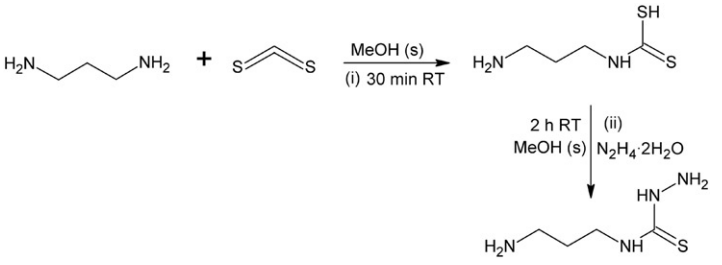
Cyclohexylamine-N-thiohydrazide and 1,3-propanediamine-N-thiohydrazide were synthesized using published methods [7–10].

2.3.1. Preparation of cyclohexylamine-N-thiohydrazide (ChaThz):[L^1]. In a round bottom flask, 0.05 mol (5.73 mL, $d = 0.86$ g cm^{-3}) of cyclohexylamine was dissolved in 20 mL methanol and carbon disulfide, 0.05 mmol (3.02 mL, $d = 1.26$ g cm^{-3}), methanol solution was added to the amine solution with constant stirring for 30 min at room temperature (RT). Hydrazine hydrate, 0.05 mmol (2.44 mL, $d = 1.026$ g cm^{-3}), methanol solution (20 mL) was added to the reaction mixture; after 2 h the product was filtered and dried in air (scheme 1). The white-yellow product was dried over $CaCl_2$ under vacuum overnight. The yield was 9.222 g (90%) and the observed m.p. was 182°C.

2.3.2. Preparation of 1,3-propanediamine-N-thiohydrazide (PdaThz):[L^2]. In a round bottom flask, 0.05 mol (4.2 mL, $d = 0.86$ g cm^{-3}) of propanediamine was dissolved in 25 mL methanol and carbon disulfide, 0.05 mmol (3.02 mL, $d = 1.26$ g cm^{-3}), methanol solution was added to the amine with constant stirring for 30 min at room temperature. Hydrazine hydrate, 0.05 mmol (2.44 mL, $d = 1.026$ g cm^{-3}), methanol solution (20 mL) was added to the reaction mixture; a white-yellow product was formed after 2 h constant stirring at room temperature (scheme 2). After filtration, the product was dried over $CaCl_2$ under vacuum overnight. The yield was 8.325 g (87%) and the observed m.p. was 164°C.

2.4. *Preparation of complexes*

$[Sn(ChaThz)_2]$ (**1**), $Sn(ChaThz)_2Cl_2$ (**2**), $[Sn(PdaThz)_2]$ (**3**), and $[Sn(PdaThz)_2Cl_2]$ (**4**) were prepared from $SnCl_2 \cdot 2H_2O$ (2.5 mmol, 560 mg) and $SnCl_4 \cdot 5H_2O$ (2.5 mmol,



Scheme 2. Synthesis for 1,3-propanediamine-N-thiohydrazide (PdaThz), L^2.

876 mg) with the corresponding ligands (34.65 mg, 5 mmol) [L^1] and (29.64 mg, 5 mmol) [L^2] in 1 : 2 molar ratios in methanol with constant stirring for 4 h under reflux. All the compounds were obtained after slow evaporation; generally the complexes were yellow. All four compounds were dried over $CaCl_2$ under vacuum overnight and characterized by spectroscopic techniques.

2.5. In vitro *antibacterial activity*

Pathogenic or opportunistic bacteria were selected for screening of the antibacterial activities of the compounds. Gram positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) which have varying cell membrane characteristics were tested. Stock cultures, kept at −80°C, were thawed at room temperature and sub-cultured in solidified nutrient agar medium, incubated at 37°C overnight and subsequently used in the experiment.

2.5.1. Disc diffusion assay. To determine the ability of ChaThz, PdaThz, **1**, **2**, **3**, and **4** to restrict the growth of selected bacteria, an initial phase of antibacterial tests were undertaken. A sterile cotton swab was used to streak bacterial culture over the entire surface of solidified Muller–Hinton agar. After 5 min at room temperature, circular discs (6 mm diameter) impregnated with various concentrations (100, 200, and 400 μg per disc) of synthesized compounds were placed on the surface of the media, and the culture plate incubated at 37°C for 24 h. Growth restriction was identified as clear zones around the discs and the result recorded as average diameter of these zones around four discs in the plate. Commercial antibiotics (vancomycin and carbenicillin) were used as positive controls.

2.5.2. Bacteriostatic and bactericidal assay. Precise assessment of bacterial susceptibility to a compound requires determination of the minimum inhibitory concentration (MIC). To determine the MIC, stock solutions of the compounds were serially diluted using standard two-fold tube dilution procedures and 2 mL of overnight culture (adjusted to an optical density of McFarland 0.5 standard) of bacteria were added to each tube. After 20 h of incubation, the MIC was determined as the lowest concentration of the compound for the tube(s) showing similar turbidity to that of the negative control (no nutrient broth added). The tubes' content was then plated on

solidified nutrient agar media and incubated overnight, after which the minimum bactericidal concentration (MBC) was determined as the highest dilution showing killing at least 99.9% of the initial bacterial inoculum.

2.6. In vitro *antifungal activity*

Sterile 6 mm test discs impregnated with L^1, L^2 **1**, and **3** were placed on cultures of fungal species (*Aspergillus flavus*, *A. carbonarius*, *A. niger*, and *A. fumigatus*) to test their inhibitory effects on fungi in this study. Amphotericin B (AmB) (20 µg per disc) was used as a reference drug for fungal inhibition. Potato dextrose agar (PDA) was prepared by dissolving 39 g of PDA powder in 1 L of sterile water and autoclaving the solution for 15 min at 121°C. The content was then cooled to 50°C and 0.05 g each of streptomycin and chloramphenicol (dissolved in 1 mL ethanol) was added.

2.6.1. Disc diffusion assay. A disc application technique of Rauter and co-workers [16] was employed *in vitro* to evaluate the antifungal activities. Mature conidia of fungal isolates were harvested from PDA plates and suspended in Ringer's solution; spore suspensions were standardized with a haemocytometer (1×10^4 conidia mL^{-1}). One milliliter conidial suspension representing each fungal isolate was then spread on a 90 mm Petri dish containing 20 mL PDA with excess conidial suspension decanted and allowed to dry. Three working solutions were prepared by dissolving 6.75, 13.5, and 27 mg of the ligands and complexes in 1 mL of DMSO and 15 µL of each complex dilution was used to impregnate a sterile 6-mm diameter test disc (AB BioDisk, Solna) at concentrations of 100, 200, and 400 µg per disc. The disc was then air-dried and individually placed diagonally on three separate points on each inoculated plate (3 replicates). Amphotericin B (20 µg per disc) (AB BioDisk, Solna) was used as a reference for fungal inhibition and DMSO was used as a negative control. Plates were incubated at room temperature (22–25°C) for 7 days. The radius of the inhibition zone of fungal growth along two axes at right angles to one another was determined on day 3, day 5, and day 7 as the percentage minimum inhibition zone (%MIZ) calculated as:

$$\%\mathrm{MIZ} = \frac{\pi r^2}{\pi R^2} \times 100,$$

where r is the difference between the radius of zone of inhibition in the presence of the test complex and that of the control and R is the radius of the Petri dish.

Minimum inhibition dose (MID) was determined as the lowest concentration of the tested complex, which showed the least visible fungal growth inhibition.

2.6.2. Statistical analysis. A one-way analysis of variance (ANOVA) was performed to derive mean values, which were compared by the least significant difference (LSD) method using all pairwise multiple comparison procedures (Holm–Sidak method) [17]. Mean values among treatment groups were deemed to have significant differences if the level of probability was ≤ 0.05.

Table 1. Physical properties of L^1 and L^2 and tin complexes.

Compound	Color	m.p. (°C)	Yield (%)	Conductivity ($\Omega^{-1}\,cm^2\,mol^{-1}$)
(ChaThz) [L^1]	White-yellow	182	90	–
(PdaThz) [L^2]	White-yellow	164	87	
[Sn(ChaThz)$_2$] (**1**)	Yellow	205	67	13.1
[Sn(ChaThz)$_2$Cl$_2$] (**2**)	Lemon-yellow	195	75	14.2
[Sn(PdaThz)$_2$] (**3**)	Yellow	237	85	13.7
[Sn(PdaThz)$_2$Cl$_2$] (**4**)	Light-yellow	222	70	14.6

Table 2. Elemental analysis data of L^1 and L^2 and complexes.

Compound	Molecular weight (Calcd)	C% obs. (Calcd)	H% obs. (Calcd)	N% obs. (Calcd)	S% obs. (Calcd)
(ChaThz) [L^1]	173	48.27 (48.52)	8.64 (8.73)	24.18 (24.25)	18.37 (18.50)
(PdaThz) [L^2]	148	32.21 (32.41)	8.25 (8.16)	37.67 (37.80)	21.79 (21.63)
[Sn(ChaThz)$_2$] (**1**)	459	37.83 (37.61)	2.79 (2.71)	18.61 (18.80)	14.55(14.34)
[Sn(ChaThz)$_2$Cl$_2$] (**2**)	534	19.98 (19.85)	4.64 (4.58)	23.57 (23.35)	13.17 (13.25)
[Sn(PdaThz)$_2$] (**3**)	409	32.03 (32.21)	3.16 (3.09)	16.24 (16.10)	12.48 (12.28)
[Sn(PdaThz)$_2$Cl$_2$] (**4**)	484	23.24 (23.49)	4.27 (4.43)	27.17 (27.39)	15.45 (15.67)

3. Results and discussion

3.1. *Physical characterization*

All complexes are soluble in DMF and DMSO. The molar conductances of the isolated complexes measured in DMSO are less than $15\,\Omega^{-1}\,cm^2\,mol^{-1}$ suggesting non-electrolytes. In table 1 colors, melting points, conductivities, and yields of all the complexes are listed and elemental analyses in table 2, signifying the stoichiometries and physical characteristics of the tin compounds.

3.2. *Electronic spectra*

Absorption bands of electronic spectra of the complexes in DMSO are listed in table 3. The thiohydrazide ligands and complexes show intense absorptions in the UV region due to the N═══C═══S chromophore. One absorption at ~250 nm is assigned to imine (C=N) n–π^* transition. The complex also exhibits a strong band at ~415 nm, which can be assigned to ligand to metal charge-transfer transition. There is significant shifting in the chromophore due to coordination with tin [18, 19].

3.3. *IR spectra*

Comparative IR spectra of the ligands and compounds are summarized in table 4. Strong bands at ~3300 cm^{-1} and 3160 cm^{-1} in thiohydrazide and thiodiamine metal complexes are assigned to −NH stretching vibrations of NH_2 and NH. The NH

Table 3. Electronic spectral data of the ligands and their complexes.

	λ_{max} (nm)	
Compound	(C=N) n–π*	Charge transfer
(ChaThz) [L^1]	240	
(PdaThz) [L^2]	224	
[Sn(ChaThz)$_2$] (**1**)	247	418
[Sn(ChaThz)$_2$Cl$_2$] (**2**)	241	438
	260	
[Sn(PdaThz)$_2$] (**3**)	247	412
[Sn(PdaThz)$_2$Cl$_2$] (**4**)	258	425

Table 4. FTIR spectra of the ligands and complexes.

Compound	ν_{N-H}	Thioamide I	Thioamide II	Thioamide III (ν_{N-N})	Thioamide IV ($\nu_{C=S}$)	ν_{Sn-N}	ν_{Sn-S}
(ChaThz) [L^1]	3301	1604	1507	1075	904	–	–
(PdaThz) [L^2]	3274	1609	1485	1100	949	–	–
[Sn(ChaThz)$_2$] (**1**)	–	1604	1511	1073	970	555	650
[Sn(ChaThz)$_2$Cl$_2$] (**2**)	3192	1588	1465	1042	923	528	655
[Sn(PdaThz)$_2$] (**3**)	3202	1610	1537	1161	936	510	609
[Sn(PdaThz)$_2$Cl$_2$] (**4**)	3161	1609	1542	1186	949	505	599

stretching band is shifted to lower frequency, from coordination of terminal NH_2 to tin. The lower frequency shift is justified as the molecule is more stable. After coordination with the ligands, the resulting compound was stable. Bands located from ~1588 cm^{-1} to 904 cm^{-1} were assigned to thioamide I, II, III, and IV vibrations, respectively. A sharp and strong band between 949 cm^{-1} and 904 cm^{-1} in IR spectra of the free ligands is shifted in tin complexes corresponding to their bonding (table 4), attributed to ν(C=S) [20]. The characteristic IR absorption frequencies for Sn–S (~600 cm^{-1}), Sn–N (~525 cm^{-1}) and their corresponding band positions were observed, confirming the Sn–N and Sn–S bonding in the tin(IV) compounds.

3.4. *^{1}H-NMR spectra*

^{1}H-NMR spectra of ligands and their metal complexes were recorded in DMSO-d_6 with TMS as internal standard.

^{1}H-NMR spectra of thiohydrazide ligands [7–10] showed signals for δ 9.68 ppm and δ 7.85 ppm, which could be attributed to the presence of NH protons which were lost due to D_2O exchange. The disappearance of the NH stretching bands shows the bonding of NH with tin(IV). Three signals at δ 2.93 ppm, δ 1.77 ppm, and δ 1.29 ppm indicate the presence of the $-CH_2$ group in the ligands (table 5). Downfield shifting and disappearance of the NH_2 signal in the resulting compounds (**1**, **2**, **3**, and **4**) have been observed in the spectra confirming the involvement of the bonding with tin(IV) (figure 1).

Table 5. ^{1}H-NMR data (ppm) for ligands and complexes.

Compound	NH_2	NH	CH_2	CH_2	CH_2
(ChaThz) [L^1]	[d]4.12(d,2H)	[c]7.85(t,H), [b]8.48(s,H)	[f]2.93(m,4H)	[a]1.77(t,4H)	[e]1.29(m,2H)
(PdaThz) [L^2]	[g]4.39(d,2H) [a]3.72(t,2H)	[f]8.37(t,2H) [e]8.25(t,2H)	[d]1.73(m,2H)	[c]2.81(dt,2H)	[b]3.53(q,2H)
[$Sn(ChaThz)_2$] (**1**)	–	7.98(s,2H)	2.91(m,2H)	1.70(t,4H)	1.28(q,2H)
[$Sn(ChaThz)_2Cl_2$] (**2**)	–	7.97(s,2H)	2.97(m,4H)	1.68(t,4H)	1.24(q,2H)
[$Sn(PdaThz)_2$] (**3**)	4.06(s,2H) 3.65(d,2H)	8.03(s,2H)	1.67(m,2H)	2.97(dt,2H)	3.58(q,2H)
[$Sn(PdaThz)_2Cl_2$] (**4**)	4.10(s,2H) 3.75(d,2H)	7.93(s,2H)	1.77(m,2H)	2.92(dt,2H)	3.64(q,2H)

Superscript "a" to "g" refers to the position of the proton in the synthesized compound.

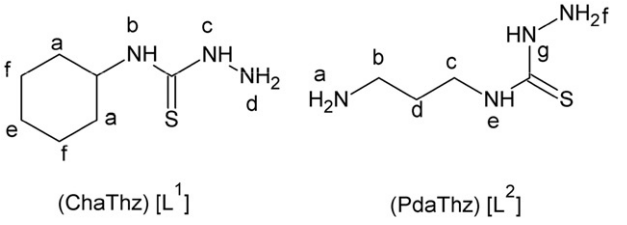


Figure 1. Structural representation of L^1 and L^2.

3.5. *^{119}Sn NMR spectra*

^{119}Sn NMR spectroscopy gives significant information to determine coordination around tin with the ^{119}Sn chemical shift depending upon the nature and orientation of the groups bonded to tin. ^{119}Sn spectra of **1** and **2** exhibit single resonances at δ −137.03 ppm possibly for tetrahedral geometry and −670.27 ppm for octahedral geometry, respectively, shifted from $SnCl_2$ and $SnCl_4$ precursors (−376.27 ppm and −624.93 ppm) due to coordination with –C(S)–$NHNH_2$. For **3** and **4**, shifts were observed at −210.45 ppm (tetrahedral) and −695.86 ppm for octahedral [21].

3.6. *Mass spectroscopy*

Mass spectra were recorded by the direct injection method of ESCi +/−. For tin having 10 naturally occurring isotopes, a series of peaks appear close to each other. Fragmentation patterns were not considered, only the molecular ion peaks for the four complexes. For [$Sn(ChaThz)_2$] (**1**), the three major peaks are m/z 454, 456, and 458 by the source of ES – for [$Sn(ChaThz)_2$]–H^+ molecular ion for the different isotopes of tin. For **2** m/z 535–550, a series of different intensity peaks were observed with maximum intensity of 541 for [$Sn(ChaThz)_2Cl_2$]–H^+ using ESCi-analysis. Complexes **3** and **4** were analyzed by ESCi-source with a series of peaks at m/z 408 and 483, respectively, for [$Sn(PdaThz)_2$]–H^+ and [$Sn(PdaThz)_2Cl_2$]–H^+ [22].

From the above spectroscopic data, the proposed structures for **1** and **3** are tetrahedral and octahedral geometry is proposed for **2** and **4** (figure 2).

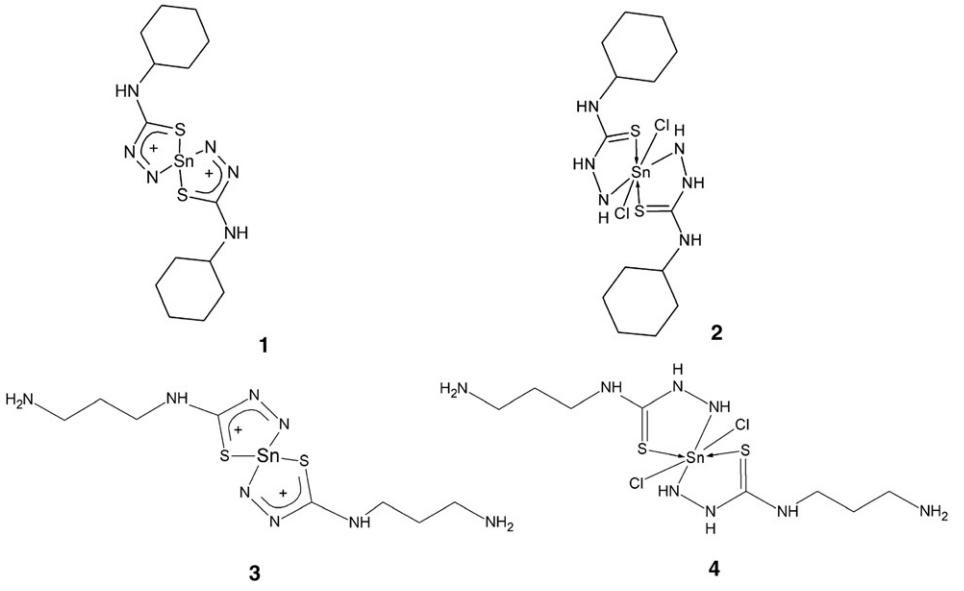


Figure 2. Compound coordination behavior and proposed structures for tin complexes.

3.7. In vitro *antibacterial study*

3.7.1. Bacterial growth inhibition. The *in vitro* antibacterial screening of the ligands and tin compounds show (table 6) inhibitory activities against selected microorganisms. Compound **1** shows better performance than any other compound, exhibiting potent antibacterial activities against all bacteria and was most active against Gram-positive bacteria; the diameter of the clear zones were 8–17 mm against *B. Cereus* and 17–20 mm against *S. Aureus*. This result corroborates the findings of other researchers [23] who found that Gram-negative bacteria were more resistant to tin compounds than Gram-positive bacteria. However, the higher activity of L^1 specifically against *S. aureus* could be ascribed to more complex inhibitory mechanisms than the common ability of ligands to affect cell membrane permeability. The inhibitory activities of **1** against bacteria were in some cases more pronounced than those obtained with the commercial antibiotic vancomycin. Compounds **2**, **3**, and **4** failed to inhibit the growth of *B. cereus* but were active against *P. aeruginosa* and *S. aureus*. In general, the ligands were not as effective as their corresponding compounds, although L^1 showed stronger inhibitory activity against *S. aureus* than any other compound.

In some cases higher concentrations of compound did not exhibit greater antibacterial activities. This can be due to the fact that for a given compound and depending on the mechanism of active elements, microorganisms can quickly anticipate danger and develop resistance, especially at relatively higher concentration of the compound, explaining the absence of significant difference between the inhibitory effect of compounds at 200 and 400 μg.

In this study, confirmation of antibacterial activities of the compounds using the disc diffusion method leads to further assessment of the minimum concentration required

Table 6. Zones of inhibition for antibacterial screening.

Complexes	Content (μg per disc)	Diameter of inhibition (mm)			
		E. coli	*P. aeruginosa*	*S. aureus*	*B. cereus*
(ChaThz) [L^1]	100	R	R	25	R
	200	7	R	30	R
	400	9	R	35	R
(PdaThz) [L^2]	100	R	R	R	R
	200	R	10	R	R
	400	9	14	R	R
$[Sn(ChaThz)_2]$ (**1**)	100	R	R	17	8
	200	9	10	19	8
	400	11	11	20	17
$[Sn(ChaThz)_2Cl_2]$ (**2**)	100	8	8	R	R
	200	10	8	11	R
	400	14	10	11	R
$[Sn(PdaThz)_2]$ (**3**)	100	R	9	11	R
	200	R	9	14	R
	400	R	11	16	R
$[Sn(PdaThz)_2Cl_2]$ (**4**)	100	R	R	11	R
	200	R	9	11	R
	400	R	9	11	R
Positive controls					
Vancomycin 30 μg		8	R	16	18
Carbenicillin 100 μg		9	27	40	10

R: Resistant.

for the antimicrobial activities and the type of inhibition (bacteriostatic or bactericidal) prevailing.

3.7.2. Minimum concentration of antibacterial activities. The MIC was determined in this study through the broth dilution method. The highest concentration considered was 200 μg per disc and absence of activity at this concentration should not be interpreted as total lack of antibacterial ability for any given compound. The MIC values (table 7) ranged between 100 and 200 μg per disc, but **3** did not show significant ability to restrict the growth and prevent increased turbidity in test tubes. The lowest MIC was recorded with **1**, implying that this compound is most effective; this finding is also reflected in the results of the disc diffusion assay. The MBC results, which allowed for the determination of whether the activities of the antibacterial compounds were bacteriostatic or bactericidal, showed that all inhibitory activities against *E. coli* were bacteriostatic, while the activities against *P. aeruginosa* were bactericidal. Compound **1** was lethal against *P. aeruginosa*, *S. aureus*, and *B. cereus* but other compounds exhibited bacteriostatic activities. Tin compounds exert their antibacterial activities by acting against mitochondria, chloroplasts, and ATPases, as well as degrading the microbial cells [24]. However, the effects of tin derivatives on microorganisms are also related to their molecular area and some microorganisms have the ability to degrade or reduce the toxicity of particular tin complexes [25, 26]. The effect of various compounds may then vary among microorganisms as the cell membrane structure that determines

Table 7. MIC and MBC of tested compounds.

	MIC ($\mu g\,mL^{-1}$)				MBC ($\mu g\,mL^{-1}$)			
Selected bacteria	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
E. coli	100	200	ND	200	ND	ND	ND	ND
P. aeruginosa	100	100	ND	100	200	200	200	200
S. aureus	100	200	200	200	200	ND	ND	ND
B. cereus	100	100	ND	100	100	ND	ND	100

ND: Not detected.

Table 8. Effects of ligand complexes on %MIZ of fungi on day 3.

		%MIZ			
Complexes	Content	*A. flavus*	*A. carbonarius*	*A. niger*	*A. fumigatus*
1	100	0.9 ± 0.1	0.44 ± 0.00	0.68 ± 0.09	1.5 ± 0.1
	200	1.0 ± 0.1	0.44 ± 0.00	1.01 ± 0.08	1.2 ± 0.2
	400	0.97 ± 0.09	0.44 ± 0.00	1.1 ± 0.1	1.4 ± 0.1
	Probability	NS	NS	NS	NS
3	100	0.97 ± 0.07a	0.70 ± 0.04a	1.09 ± 0.09a	1.6 ± 0.2
	200	1.1 ± 0.1a	0.83 ± 0.04ab	1.12 ± 0.05a	1.64 ± 0.09
	400	1.6 ± 0.1b	0.93 ± 0.04b	2.0 ± 0.4b	1.6 ± 0.2
	Probability	***	**	*	NS
L^1	100	5.3 ± 0.2a	3.2 ± 0.1a	9.9 ± 0.8a	10.3 ± 0.6a
	200	8.3 ± 0.6b	5.6 ± 0.7b	15.3 ± 2.2b	13.2 ± 0.6b
	400	7.9 ± 0.4b	6.0 ± 0.6b	21.0 ± 1.9b	16.00 ± 0.00c
	Probability	***	**	**	***
L^2	100	1.0 ± 0.1a	0.93 ± 0.04	1.28 ± 0.08a	1.41 ± 0.05a
	200	1.5 ± 0.1b	1.24 ± 0.00	1.5 ± 0.2ab	1.64 ± 0.06ab
	400	1.6 ± 0.09b	1.7 ± 0.5	2.3 ± 0.7b	2.6 ± 0.2b
	Probability	**	NS	***	***

Values within columns are means (left) and standard error of the mean (right).
NS: not significant, MIZ: minimum inhibitory zone.
a,b,c: Mean values in the same column for each complex not sharing the same letters are significantly different.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

the penetration of the compound into the cell may differ between species or genera, and the metabolic activity is also not identical for all microorganisms.

3.8. In vitro *antifungal activity*

Antifungal activities of tin complexes against *Aspergillus* spp. over time were studied *in vitro* and results based on %MIZ are summarized in tables 8–10; comparisons made with tested complexes and the reference drug, amphotericin B, against similar fungal species are shown in tables S1, S2, and S3. The data are presented as %MIZ and MID ranges. DMSO was used as a negative control and has no inhibitory effect (%MIZ of 0.44) on the tested fungi.

Applications of thiohydrazides, thiodiamines, and thiohydrazones have been due to their significant antimicrobial activities [27–29]. In this study, data reveal the potency of

Table 9. Effects of ligand complexes on %MIZ of fungi on day 5.

Complexes	Content	%MIZ			
		A. flavus	*A. carbonarius*	*A. niger*	*A. fumigatus*
1	100	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	200	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	400	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	Probability	NS	NS	NS	NS
3	100	0.44 ± 0.00a	0.44 ± 0.00	0.44 ± 0.00a	0.44 ± 0.00
	200	0.94 ± 0.09b	0.44 ± 0.00	0.94 ± 0.09b	0.44 ± 0.00
	400	1.09 ± 0.09b	0.44 ± 0.00	0.63 ± 0.13b	0.44 ± 0.00
	Probability	***	NS	**	NS
L^1	100	1.2 ± 0.2a	0.76 ± 0.06	6.3 ± 0.7a	3.5 ± 0.5a
	200	1.8 ± 0.1b	1.3 ± 0.2	13.0 ± 2.3b	7.0 ± 0.6b
	400	2.1 ± 0.3b	1.3 ± 0.1	18.0 ± 2.1b	10.6 ± 1.3c
	Probability	***	NS	**	***
L^2	100	0.56 ± 0.07a	0.62 ± 0.08a	0.44 ± 0.00a	0.44 ± 0.00a
	200	1.0 ± 0.1b	0.79 ± 0.00b	0.53 ± 0.06a	0.79 ± 0.00b
	400	1.0 ± 0.2b	0.79 ± 0.00b	0.79 ± 0.00b	0.86 ± 0.07b
	Probability	*	*	**	***

Refer notes of table 8.

Table 10. Effects of ligand complexes on %MIZ of fungi on day 7.

Compound	Content	%MIZ			
		A. flavus	*A. carbonarius*	*A. niger*	*A. fumigatus*
1	100	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	200	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	400	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	Probability	NS	NS	NS	NS
3	100	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	200	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00	0.44 ± 0.00
	400	0.44 ± 0.00	0.44 ± 0.00	0.8 ± 0.1b	0.44 ± 0.00
	Probability	NS	NS	NS	NS
L^1	100	2.6 ± 0.4	1.20 ± 0.09	2.1 ± 0.3a	1.4±0.2a
	200	3.2 ± 0.5	1.2 ± 0.1	4.0 ± 0.9ab	2.6 ± 0.3b
	400	4.0 ± 0.5	1.4 ± 0.3	7.1 ± 1.4b	5.7 ± 0.8c
	Probability	NS	NS	**	***
L^2	100	0.50 ± 0.06a	0.56 ± 0.07a	0.44 ± 0.00a	0.44 ± 0.00a
	200	0.9 ± 0.1b	0.8 ± 0.6b	0.44 ± 0.00a	1.1 ± 0.1b
	400	1.1 ± 0.08b	1.01 ± 0.08c	1.09 ± 0.09b	1.20 ± 0.04b
	Probability	***	***	***	***

Refer notes of table 8.
**$p < 0.01$, ***$p < 0.001$.

some of the tested compounds to suppress fungal growth with maximum activity exhibited on all fungal isolates throughout the experimental period with L^1. Conversely, **1** was least effective with no activity observed in a culture of *A. carbonarius* on day 3 even at 400 µg per disc (table 8) and against all isolates from days 5 to 7 (tables 9 and 10). For other fungal isolates including *A. flavus*, *A. niger*, and *A. fumigatus*,

MID of this compound was 100–200 μg per disc. The low activity of complexes in this study may be due to the fact that *Aspergillus* spp. has a hard chitin layer in the outer wall and therefore requires higher levels of these compounds to kill the fungi as suggested by Mishra and Kaushik [8]. With increasing dose levels of the tested complexes, antifungal activity for the most part was enhanced. In a few circumstances, inconsistent data on %MIZ was observed in this study either with increasing application dosage or over time as seen in some studies [14–16, 30]; these data could not be explained with high degree of certainty but may be related to either the hydrophilic nature of some micro-organisms during growth or due to the higher permeation potential of the diluted compound into the fungal cell wall.

L^1 is most potent, exhibiting high potential fungistatic activity with significantly ($p < 0.001$) much higher MIZ above 13% on average, particularly in cultures of *A. niger* and *A. fumigatus*, when compared with other complexes. Relative to L^1, AmB (used as a reference drug) had lower MIZ of 8.6% and 4.8% in *A. niger* and *A. fumigatus* cultures, respectively (table S1). L^2 had lower antifungal activity when compared to L^1 but its activity was higher than those of the complexes. The mechanism through which these compounds exhibit their antifungal activity might be related to the presence of thiocarbohydrazide [31], which may inhibit chitin synthesis in fungi required for the protection of the fungal cell and its constituents.

MID of L^1 was achieved within 10–20 μg per disc range on day 3 and was maintained until day 7, except in the case of *A. carbonarius* where the MID range increased to 60 and 80 μg per disc. L^2 showed moderate activities which increased proportionately in all fungal cultures from MID of 40 to 60 μg per disc recorded on day 3 to 60–100 μg per disc and 80–100 μg per disc (100–200 μg per disc for *A. niger*) on days 5 and 7, respectively (tables 9, S1, and S3). One important consideration when choosing between various antifungal therapeutic drugs is whether the chosen drug has long-lasting potential. Herein, the prolonged potential activities of L^1 and L^2 against the tested fungal species were more pronounced and long-lasting than those of the tin(IV) complexes including **1** and **3** against similar fungal species. Antifungal activities of L^1 and L^2 were exhibited in all cultures, while growth of all fungi was not affected by **1**. Compound **3** showed some activity against *A. flavus* ($p < 0.001$) and *A. niger* ($p < 0.01$) at higher application levels (200 and 400 μg per disc), table 8. If used in therapy, such compounds may be applied as a single dose, thus minimizing possible toxic effects in humans due to their long-lasting abilities.

On day 5, L^1 showed significantly higher %MIZ in cultures of all fungi used than for other compounds at $p < 0.001$ (table 9), with similar findings observed on day 7 (table 10). The potential of compounds to inhibit fungal growth was reduced considerably from days 3 to 7. However, the activities of L^1 and L^2 were long-lasting in inhibiting growth of all the fungi and thus may be of interest and could be considered as potential antifungal agents, especially as these antifungal activities were similar or even better than those of AmB which is commonly used in the treatment of systemic fungal diseases in humans. The mechanism of action of compounds against fungi may in part involve breaking down the cell wall [7], DNA damage [28], and inhibition of protein synthesis or may be due to their ability to chelate with metal ions in the cell and deprive it of the needed ions [32, 33]. L^1 at extremely low doses (MID 10–20 μg per disc) as found herein, was regarded as the most potent even when compared to other compounds previously tested under similar conditions [34] and thus, may be applied as

a broad-spectrum antifungal agent or a component of it against fungal infectious diseases.

4. Conclusion

Electronic, IR, and NMR spectral studies indicate that metal–ion complexation taking place is through azide nitrogen and thioamide sulfur; elemental analyses show stoichiometry of the compounds with proposed tetrahedral and octahedral geometry. The complexes are readily soluble in DMF and DMSO. Molar conductance values in DMSO indicate non-electrolytes. Preliminary antimicrobial screening shows good results especially for L^1. The antibacterial and antifungal activities of ligands and the complexes are due to functional group (–C(S)–NH–NH_2). The activities are sometimes better in ligands because these groups are free and working as cell wall breaking mechanisms to kill or at best inhibit the growth of microorganism. In the case of the complexes, coordinating groups are not available as in the ligands due to coordination with tin(IV) causing less inhibition on the microorganism. Trials to obtain crystals suitable for structure determination by X-ray crystallography were in vain due to the amorphous nature of the complexes. Based on spectroscopic studies, the structures of the complexes are proposed as depicted in figure 2.

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